ON THE DIFFERENCE IN CONTENT OF AGGLUTININS IN BLOOD SERUM AND PLASMA.[1]

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IN following up the consideration of the subject discussed in our earlier paper (1909[1]), namely, the origin of immune substances, it appeared to us that if the hypothesis which attributes their production to the activities of the leucocyte-forming tissues were correct, important evidence might be obtained by a comparison of blood serum and plasma during the process of active immunisation.

Many of the results obtained by previous investigators who have endeavoured to compare the serum and plasma of an animal have been irregular and contradictory. And where no differences have been found to exist between the fluids in question, the methods used have not always been sufficiently precise to reveal such differences, unless they had been of a very high degree. Especially is this the case since most of the recorded observations are based on a determination of the bactericidal action of the fluids examined.

Hahn (1895[2]), who prepared his plasma by the addition of histon to the blood, found no difference between the bactericidal power of serum and plasma, and concluded that the bactericidal substances circulate in the plasma. Sawtchenko (1893[3]), comparing serum and leech-extract plasma, reached a similar conclusion. But the observations of Gengou (1901[4]) with non-clotting goose's plasma, in which the bacteriolytic power was practically nil, show that these experiments only concern the question of the origin of complement which we have dealt with in a previous paper (1909[1]). Similar objections apply to the results of Hewlett (1903[5]), and Bellei (1904[6]).

It therefore seemed to us desirable to carry out a close investigation of the serum and plasma as regards their content of agglutinins, since agglutinins can be measured with extreme exactness and precision.

Of previous observers, von Löwit and Schwarz (1903[7]), who studied natural agglutinins, obtained different results with differently prepared plasmas. And in leech-extract plasma they sometimes found the agglutinins stronger and sometimes weaker than in the corresponding samples of blood serum. Figari (1904[8]), on the other hand, who worked on specific tubercle agglutinins, always found his centrifugate plasma very much weaker than his coagulation plasma, concluded that agglutinins do not exist free in the blood plasma.

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Methods.

In all our experiments the plasma which we used was leech-extract plasma. No antiseptics were employed to preserve the fluids, which remained sterile for long periods in the cold chamber. The general plan of our experiments was as follows. Rabbits were taken and each received a small intraperitoneal injection of dead *Bacillus coli.* Both before, and for a period following, this inoculation blood was taken daily from the marginal ear vein into two sterile tubes. The one sample—for serum—was allowed to clot in the usual way, and the serum separated on the following day. The other—for plasma—was received into a sterile tube containing a little dry leech extract; it was rapidly centrifugalised, and the plasma pipetted off and stored in a sterile tube. All the tubes were closed with sterilised rubber stoppers and kept in a cold chamber until required for use. Control experiments showed that the leech extract added did not affect the agglutinating power of the fluids.

As regards the centrifugalisation of leech-extract blood for the preparation of plasma, it was important to complete this as rapidly as possible so as to allow a minimum of opportunity for leucocytes to break up and discharge their contents into the fluid plasma. The centrifuge employed was a very powerful machine by Burmeister and Wain, which records its revolutions automatically. It was not found necessary in these experiments to run it above 7000 to 7200 revolutions per minute.

The time occupied in running up the speed to 7000 revolutions was about four minutes, the apparatus was held at this speed for three minutes, and ten minutes were required for it to come to rest after switching off the current. The minimum time from pricking the rabbit's ear to the separation of the supernatant plasma was therefore something like twenty-five minutes. On the average it was probably about half an hour.

The plasma thus obtained was perfectly clear, and remained unclotted for two months and more in the cold chamber. The fact that it did eventually clot if kept long enough was evidence that some part of the leucocytes of the blood, though probably only a very small proportion, had broken up during the time required for its preparation. Serum and plasma having been prepared daily from a particular rabbit for a number of weeks, the whole series of samples was examined for agglutinating action by Dreyer's modification of Madsen's original method, and the results recorded. An observation on the serum and plasma of thirty successive days entails between three and four thousand measurements with the pipette, and three separate readings of about one thousand agglutination tubes; but the method is much the most accurate that has been devised.

Each tube reading leads to a particular figure in a table. And the reciprocal of this figure multiplied by a figure representing the dilution of the serum used gives the amount of agglutinin present

per c.c. of the serum expressed in arbitrary units. Only when these calculations have been completed does one become aware of the character of the results which one has been recording. Thus any personal bias is automatically eliminated from the observations.

RESULTS.

The first point which stands out clearly in all our experiments is the fact that after the agglutinins have reached their maximum in the reaction of the animal, and are falling, the plasma is *invariably* and markedly stronger than the serum in agglutinating power. This constant and quite unequivocal difference, it appears to us, can only be attributed to an "absorption" of agglutinin from the serum by the corpuscles and solid fibrin of the clot during the time that the two remain in contact, similar to the absorption which takes place on the addition of any solid or semi-solid matter to fluids containing agglutinins or other immune-substances. This point is fundamental to the interpretation of the rest of our results.

This being admitted, then, it evidently follows that if, at any period, the serum is found on an equality with the plasma as regards agglutinating power, and much more if it is actually found to be the stronger, it must have received a considerable accession of agglutinin from some definite source which is by its nature not available for the plasma. And the only probable source, so far as we can see at present, which would fulfil the conditions, is to be found in the leucocytes of the blood.

Let us accept for a moment the hypothesis that anti-bodies are produced by the leucocytes and leucocyte-forming tissues of the body (notably the bone marrow), and are discharged by them into the blood by an active process comparable to secretion, as well as liberated by them when they disintegrate. It will follow from this that during the active period of immunisation the serum will be stronger than the plasma by just so much agglutinin as was contained in the leucocytes which have disintegrated in it (but which were removed from the corresponding plasma), less the amount of agglutinin absorbed from the serum by its clot. And this difference should be most in evidence during the early stages of the process, when there exists a marked leucocytosis in the circulating blood.

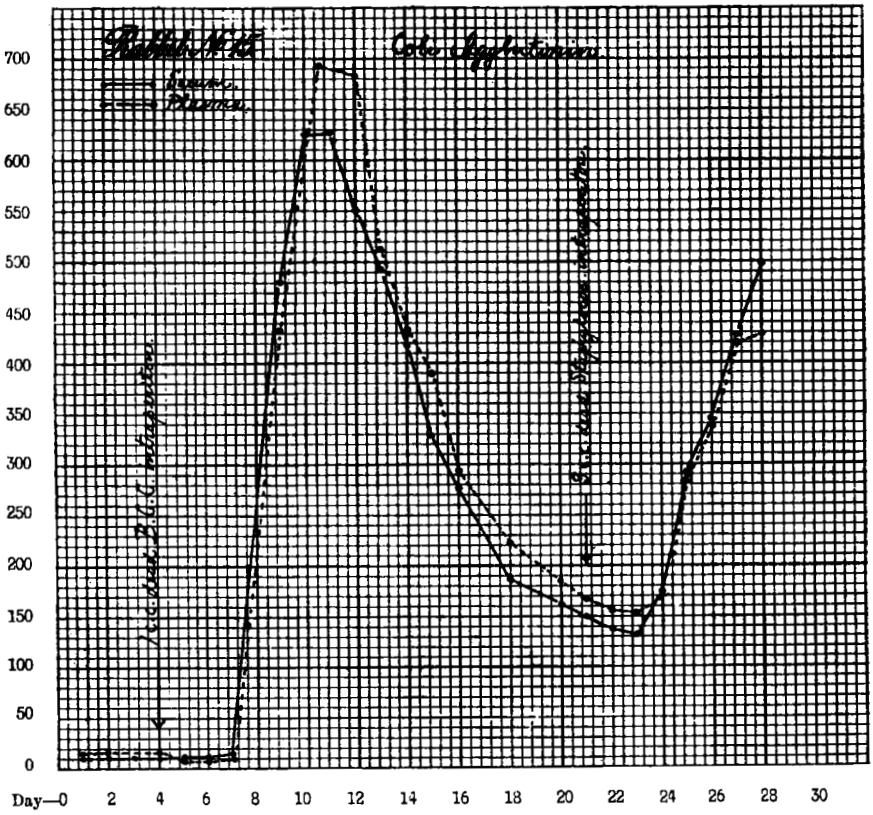
That is to say, the increase of agglutinins due to breaking up of leucocytes in the serum tube may be great enough to balance, and even more than counterbalance, the loss due to absorption by the red corpuscles and fibrin during coagulation and subsequently. These theoretical conclusions are in precise agreement with the facts we have observed in our actual measurements of the agglutinins.

In a fresh normal rabbit before inoculation we find that the

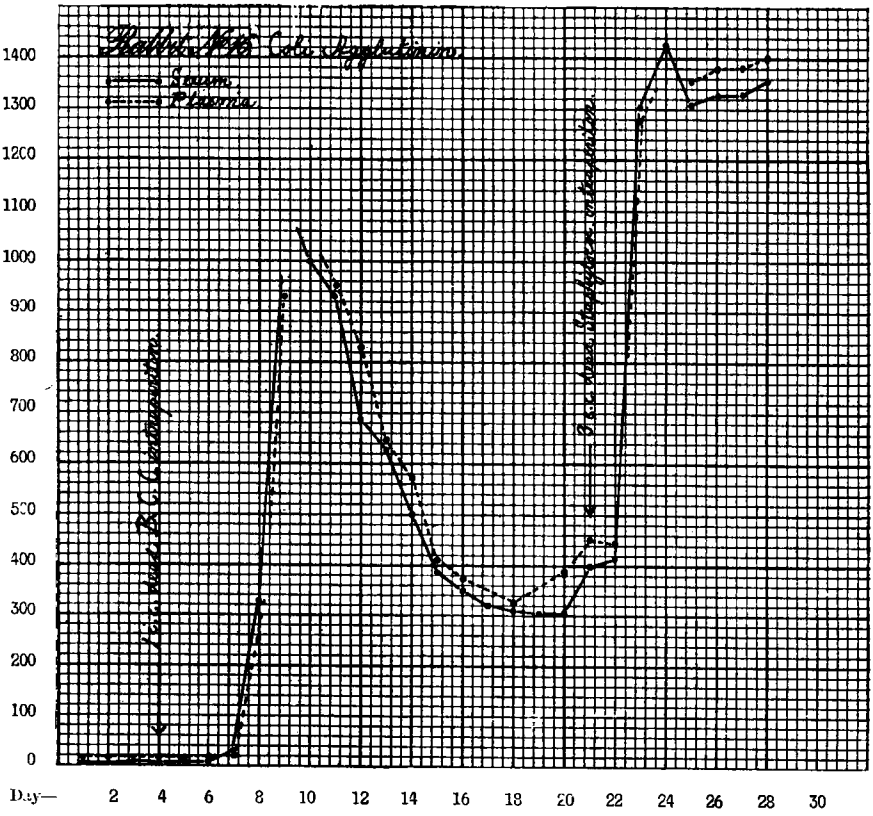
plasma is slightly more agglutinative than the serum (absorption by clot), but immediately the active production of agglutinins begins the serum shows a distinctly stronger action, and continues to be the stronger throughout the period of rise until almost the summit of the curve is reached. Here absorption begins to gain the upper hand, and from this point onwards the plasma shows the higher agglutinating power.

Our results are exhibited in the following curves, which are drawn to scale.

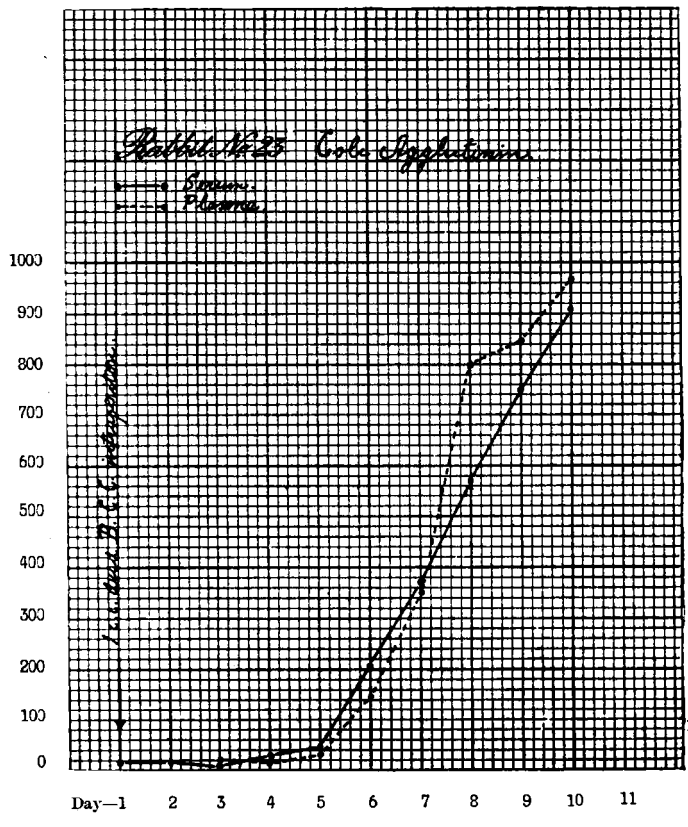
CURVE 1.


Cole Agglutinins.
Serum.
Plasma.
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
Day—0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30

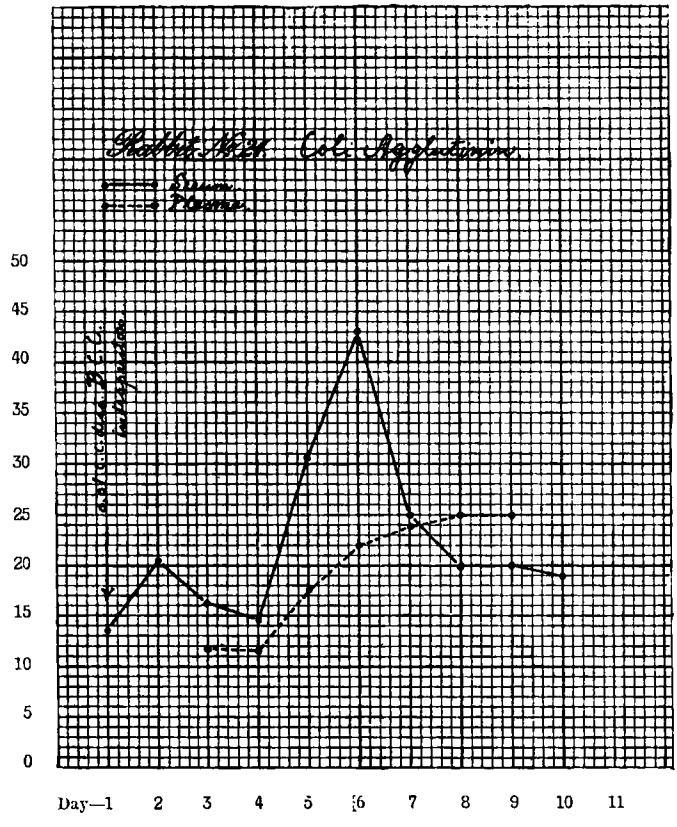
CURVE 2.


Rabbit No. 6 Col. Septicæmia.
Serum
Plasma
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
Day—
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30

CURVE 3.


Rabbit No. 23 Cobe Agglutinins.
Serum.
Plasma.
Agglutinating Power.
1000
900
800
700
600
500
400
300
200
100
0
Day—1 2 3 4 5 6 7 8 9 10 11

CURVE 4



CURVE 5.

We regard the foregoing observations as affording strong evidence that the hypothesis provisionally put forward above is justified, and that, so far as concerns the agglutinins at any rate, we have now an adequate basis for the view that the production of anti-bodies is in some way dependent on the activity of the leucocytic tissues of the body.

The plasma yields us just so much agglutinin as has been poured into it from the leucocyte-forming tissues (and possibly from elsewhere); the serum shows, in addition to this amount, the agglutinin given up after the blood was shed by the leucocytes which happened to be present in the particular blood sample taken—less the amount of agglutinin "absorbed" from it by its clot.

Attention must be drawn to a further feature of our results. If the curves and the actual tables given are carefully examined, it is at once evident that the percentage difference between the readings for the serum and plasma is neither constant nor does it follow any very regular course in our observations. This is exactly what might be expected if the leucocytes are concerned in giving off agglutinins

TABLE OF OBSERVATIONS (*Agglutinins expressed in Arbitrary Units*).

Day.	RABBIT No. A. 1. Units of Agglutinin per c.c. In Serum.	In Plasma.	Day.	Units of Agglutinin per c.c. In Serum.	In Plasma.	Day.	RABBIT No. 15. Units of Agglutinin per c.c. In Serum.	In Plasma.	Day.	RABBIT No. 16. Units of Agglutinin per c.c. In Serum.	In Plasma.	Day.	RABBIT No. 23. Units of Agglutinin per c.c. In Serum.	In Plasma.
1	8·8	9·7	37	1467	...	1	10·5	10·8	1	11	11·0	1*	19·6	...
2	9·0	9·2	38	1500	...	2	10·5	10·8	2	11	11·6	2	19·0	...
3*	8·5	...	39	1485	...	3	10·5	...	3	13·5	...	3	13·4	14·1
4	10·4	10·6	40	1500	...	4*	10·6	10·5	4*	12	12·2	4	30·5	17·7
5	31	26	41	1500	...	5	10·6	9·3	5	12	13	5	42·3	32·3
6	278	238	42	1467	...	6	10·6	9·3	6	12	13	6	203·7	147
7	...	...	43	1467	...	7	13·8	13·5	7	34	30	7	370	357
8	1579	1254	44	1467	...	8	...	...	8	333	...	8	577	800
9	1666	1500	45	1485	...	9	480	433	9	...	930	9	750	845
10	1875	1937	46	1540	1612	10	625	...	10	1000	...	10	909	967
11	1819	1876	47	1540	1612	11	627	692	11	930	952			
12	1764	1820				12	551	684	12	691	833			
13	1579	1710				13	494	512	13	627	645			
14	...	...				14	419	433	14	500	576			
15	1364	1429				15	329	390	15	384	408			
16	1111	1154				16	277	294	16	352	378			
17	1000	1037				17	...	...	17	320	...			
18	...	...				18	187	223	18	312	323			
19	833	937				19	...	...	19	307	...			
20	...	...				20	163	186	20	312	391			
21†	811	909				21‡	150	167	21‡	400	454			
22	...	...				22	139	157	22	416	444			
23	1037	1018				23	133	153	23	1304	1277			
24	1072	...				24	176	...	24	1428	...			
25	1112	...				25	292	285	25	1312	1358			
26	1464	...				26	343	340	26	1333	1382			
27	1304	1500				27	428	420	27	1333	1382			
28	...	...				28	499	429	28	1358	1405			
29	1154	...												

RABBIT No. 24.

Day.	Units of Agglutinin per c.c. In Serum.	In Plasma.
1§	13·7	...
2	20·4	...
3	16·2	11·9
4	14·7	11·6
5	30·6	17·7
6	43	22
7	25	24
8	20	25
9	20	25
10	19	...

* 1 c.c. dead *B. coli communis* intraperitoneally.
† 1 c.c. dead staphylococcus intraperitoneally.
‡ 3 c.c. dead staphylococcus intraperitoneally.
§ 0·01 c.c. dead *B. coli* intraperitoneally.

when they disintegrate. Considerable variations in the extent to which the leucocytes break up before the plasma can be separated from them must necessarily occur, in view of the unavoidable variations in the room temperature from day to day, in the time taken up in carrying out the required manipulations, and in the condition of the blood itself in an animal undergoing immunisation and also being bled daily to the amount of 6 or 7 c.c.

On the whole, however, the percentage difference (which may exceed 25 per cent.) is greatest in the earlier part of the rise. This period corresponds with the period of maximum leucocytosis following inoculation, as shown in the curves worked out by T. E. Holmes (9) in 1902 in the laboratory of one of us (E. W. A. W.) at Guy's Hospital, and confirmed by hitherto unpublished observations recently made in the Department of Pathology, Oxford, by Dr. Ray.

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